bio-protocol Preprint

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scrambled RNPs

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An abbreviated version of this protocol was published in eLIFE in Jan 2021

A simple and effective F0 knockout method for rapid screening of behaviour and other complex phenotypes

DOI: 10.7554/eLife.59683

Detailed protocol

Hi Sanmitha,

Sounds like a great project!

I would indeed recommend injecting the control larvae with scrambled gRNAs as this controls for any unspecific effects of the injection, such as physically penetrating the eggs and the presence of Cas9/RNA during early development.

To be clear: we have called these gRNAs "scrambled" in the eLife article but I realise now that this is not the best terminology. Many people (understandably) read "scrambled" as a shuffled version of the 'real' targeting gRNAs. We have used the same set of three scrambled gRNAs throughout the work, so that's not exactly correct. It would have been better to call them 'non-targeting', and we will do so in the future. I do not think it is necessary to use different scrambled/non-targeting gRNAs for each gene.

You can prepare these non-targeting gRNAs in exactly the same way as the targeting gRNAs, following dx.doi.org/10.17504/protocols.io.5qpvo52wdl4o/v3.

As for the sequences, you are welcome to buy the same as in the eLife paper. These were simply the 'negative control crRNA' #1, #2, #3 from IDT (https://eu.idtdna.com/pages/products/crispr-genome-editing/alt-r-crispr-cas9-system, under Enhancers & controls). You can manually check on the IDT website** or CHOPCHOP that these do not have any target in the zebrafish genome.

I realised recently that negative control crRNA #2 has some off-targets with 3 mismatches in protein-coding sequences (no off-target with less than 3 mismatches). I do not think this is particularly concerning (our sequencing data Figure 2F seems to indicate that off-targets with 3 mismatches do not get mutated), but just to be safe, I replaced negative control crRNA #2 by:

TAGAGCGGCTCGGTCCGGTA

Which I created simply by shuffling the sequence of negative control crRNA #3.

In summary, what I use currently is:

non-targeting crRNA #1 = CGTTAATCGCGTATAATACG
non-targeting crRNA #2 = TAGAGCGGCTCGGTCCGGTA
non-targeting crRNA #3 = GGCGCGTATAGTCGCGCGTA

I hope that helps. Let me know if you need more advice (f.kroll.17@ucl.ac.uk). Good luck!

** copy-paste their sequences from the eLife article supplementary file 1 https://cdn.elifesciences.org/articles/59683/elife-59683-supp1-v1.xlsx in Check your own design)

Francois

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Kroll, F. and Rihel, J. (2022). scrambled RNPs. Bio-protocol Preprint. bio-protocol.org/prep1869.
2. Kroll, F., Powell, G. T., Ghosh, M., Gestri, G., Antinucci, P., Hearn, T. J., Tunbak, H., Lim, S., Dennis, H. W., Fernandez, J. M., Whitmore, D., Dreosti, E., Wilson, S. W., Hoffman, E. J. and Rihel, J.(2021). A simple and effective F0 knockout method for rapid screening of behaviour and other complex phenotypes. eLIFE. DOI: 10.7554/eLife.59683